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Extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor* with improved fatty acid profile due to ultrasound assisted or pressurized liquid extraction

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ABSTRACT

Extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor* were obtained by ultrasound-assisted extraction (UAE) and pressurized-liquid extraction (PLE) using ethanol (E) or ethanol:water (E:W). Extraction yield, fatty acid profile, nutritional impact and cholesterol content were determined and compared with the initial insects. The highest extraction yield corresponded to PLE-*T. molitor* extracts. A decrease in total saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents, and an increase in the total polyunsaturated fatty acid (PUFA) content were observed for both UAE-E:W insect extracts, due to an enrichment in linoleic acid. The lipid indices (PUFA/SFA ratio, atherogenic and thrombogenic indices) for both UAE-E:W extracts were significantly improved compared with the initial insects. Although either extraction procedure led to cholesterol enrichment, the UAE-E:W conditions favoured the lowest concentration. Therefore, insects extracts with improved fatty acid profile can be selectively obtained, being UAE-E:W conditions preferred from the nutritional point of view.

Keywords: *Acheta domesticus*; *Tenebrio molitor*; edible insects; ultrasound assisted extraction; pressurized liquid extraction; fatty acids; cholesterol

1. Introduction

It is currently well known that the consumption of insects has been proposed as a sustainable alternative to palliate the demand of protein food products of animal origin, so efforts are being made to potentiate the insect-based food products. In this sense, one of the most important advances in Europe came from the modified Novel Food Regulation at the beginning of 2016 and applicable since January 2018 (European Parliament and Council of the European Union, 2015). In this modified regulation, the whole insects and their parts were included in the category of novel foods. Additionally, in 2015, the European Food Safety Authority (EFSA) contributed with a scientific opinion about insect intake and suggested a list of insect species with high potential for being used as foods for both human and animal feeds (EFSA Scientific Committee, 2015). In fact, several of these species are now authorized in the later European Regulation for animal feed for aquaculture, as it is the case of the species *Acheta domesticus* and *Tenebrio molitor* (European Parliament and Council of the European Union, 2017).

However, despite these advances, the human consumption of insects is still unpopular and not culturally acceptable in most of the developed countries. Most of the commercial insect presentations are being developed as whole insects or insect flours for being included in food products, mainly claimed as protein sources. Nevertheless, the exploration of other alternative forms of insect presentations for human consumption, and rich in other diverse compounds different to proteins, has been scarcely considered. In this sense, the production of specific insect extracts, might lead to concentrated forms of insects, rich in diverse compounds of potential interest different to protein, such as fibres, lipids or minor compounds, which might be worthy to study to potentiate the insect-based food products. In this regard, in addition to

protein, lipids are also the main component of insects, a source of energy and essential fatty acids. Insects have a high and variable fat content, being the second major component after proteins, with a range of 5-74% expressed in dry weight (Bessa, Pieterse, Sigge, & Hoffman, 2018). Therefore, the production of insect extracts under specific conditions, especially by the use of less polar solvents, might lead to the production of extracts with a concentrated amount of lipids. Additionally, concerning the fat content of insects, this percentage is higher during the larvae or pupae stages when compared with their adult stage, therefore many insect species are traditionally consumed in their larval form due to their higher energy density (Durst, Johnson, Leslie, & Shono, 2010). In general, the fat of insects has a high content of unsaturated fatty acids, especially linoleic acid (ω-6), α-linolenic acid (ω-3) and oleic acid (ω-9), although some species also show a remarkable content in saturated fatty acids, especially palmitic and stearic acids (Rumpold & Schlüter, 2013). Additionally, the lipid fraction of insects, as for most animal foods, has relevant content of minor fat-soluble components with nutritional and health implications, such as cholesterol or fat-soluble vitamins (Finke, 2013; Ramos-Bueno, González-Fernández, Sánchez-Muros-Lozano, García-Barroso, & Guil-Guerrero, 2016; Rumpold & Schlüter, 2013; Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014).

Concerning natural extracts, in general, the most traditional techniques used for the production of extracts are Soxhlet, maceration and hydrodistillation. In the case of insects, the Soxhlet technique has been described to obtain extracts from different species, such as *Acheta domesticus, Henicus whellani* (Orthoptera), *Tenebrio molitor*, *Alphitobius diaperinus, Zophobas morio* (Coleoptera), *or Blaptica dubia* (Blattodea) (Musundire, Zvidzai, Chidewe, Samende, & Manditsera, 2014; Tzompa-Sosa et al., 2014; Yi et al., 2013). However, the use of conventional techniques for natural extracts

appears to be disadvantageous, due to its time-consuming, high purity solvent requirements and low selectivity and extraction efficiency. Nowadays, these disadvantages could be solved with alternative novel techniques, such as ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), microwaves extraction or supercritical fluid extraction (Azmir et al., 2013). However, the use of these alternative techniques for the extraction of insects has been scarcely explored. For instance, microwaves were used for the extraction of phenolic compounds from *Holotrichia parallela* (Liu et al., 2012) and UAE was employed to obtain oil from *Clanis bilineata* (Lepidoptera) (Sun et al., 2018). Concerning these alternative techniques, it is interesting to remark that their use to obtain extracts from matrices with a high lipid content, as some edible insects would be, might also lead to additional advantages, such as higher extraction yields, or the production of lipid-rich extracts with modified or improved fatty acid profile when compared with the raw material. This last effect is mainly due to the versatility of organic solvents of different polarities that can be used by these methods. As an example, Otero, Quintana, Reglero, Fornari, & García-Risco (2018) recently showed that PLE extracts from algae were richer in long chain unsaturated fatty acids when they were extracted with ethyl acetate, compared with ethanol or acetone. Similarly, Castejón & Señoráns (2019) obtained higher omega-3 concentrates from algae thanks to PLE extraction with hexane compared with the traditional method of solid-liquid extraction of lipids. Otero et al. (2018) concluded that PLE might be useful as a selective technique for the extraction of specific carbon number fatty acids. According to these findings, and taking into account that some edible insects have a high lipid content that combines both high levels of unsaturated and saturated fatty acids and cholesterol, the evaluation of the impact of UAE and PLE on the lipid profile of insect extracts might be of interest. To the best of our knowledge,

no assessment has been done so far on the effect of these two alternative techniques on insect extraction of popular edible insects, such as *A. domesticus* and *T. molitor*.

Therefore, taking into account that the production of insect extracts under specific conditions might lead to lipid-rich extracts, but with variable fatty acid profiles depending on the conditions of extractions, the aim of the present study was the characterization of the fatty acid profile, the evaluation of its nutritional impact and the quantification of the cholesterol content of insect extracts obtained by advanced methods of extraction. Thus, extracts from *A. domesticus* and *T. molitor* by UAE and PLE using ethanol or ethanol:water were obtained and a comparative study with the lipid characterization of the initial insects was performed.

2. Material and Methods

2.1 Raw materials and sample preparation

A. domesticus (adult) and *T. molitor* (larva), were purchased under frozen conditions in a local company specialized in insect production intended for animal feed (Animal Center SL, Valencia, Spain). Afterwards, insects were gently rinsed with distilled water, freeze-dried (LyoBeta 15, Telstar, Terrasa, Spain), ground (Knife Mill Grindomix GM 200, Retsch GmbH, Haan, Germany), and stored at -20ºC protected from oxygen, light and moisture until further use.

2.2 Chemicals

N,O-bis-(trimethylsilyl)trifluoroacetamide (15238), linoleic acid methyl ester mix cis/trans (10 mg/mL in methylenechloride, 1 mL) (CRM47791), cis-5,8,11,14,17-eicosapentaenoic acid methyl ester (10 mg/mL in heptane, 1mL) (CRM47571), cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester (10 mg) (D2659), FAME mix GLC-

20 (1892-1AMP) were purchased from Sigma-Aldrich (Madrid, Spain). Ethanol (131086.1214) was obtained from PanReac AppliChem ITW Reagents (Barcelona, Spain). Hexane (6752-25), methanol (6712-25) and chloroform (6754-25) were obtained from Macron Fine Chemicals™ (Gliwice, Poland). Sodium hydroxide (137020) was purchased from Millipore (Burlington) and anhydrous diethyl ether (P0441021) from Carlo Erba (Milan, Italy). All solvents used in this study were high-performance liquid-chromatography or analytical grade, and the water was distilled and processed through an ultrapure water system (Milli-Q Integral 3 Water Purification System, Millipore, Burlington, MA, USA).

2.3 Obtention of the extracts

The lyophilized and ground samples were submitted to UAE or PLE with two solvents of different polarity: ethanol (E) and ethanol:water (E:W; 1:1, v/v), at a sample/solvent ratio of 1:10 (w/v). All extractions were made in duplicate.

2.3.1 Ultrasound-assisted extraction

Extractions of 2 g of insect sample were carried out in an ultrasonic equipment (Branson, SFX 550 Digital Sonifier, Brandson Ultrasonics, USA) with a sonication amplitude of 89.9 μm (equivalent to 60%), in continuous pulse by direct sonication at 20 kHz, as described by Navarro del Hierro et al. (2018). The extraction was carried out for 15 min at room temperature, and the average final temperature reached during the sample extraction was approximately 70ºC. Then, samples were centrifuged at 4500 rpm for 10 minutes and the supernatant was dried using a rotary vacuum evaporator. In the case of samples extracted with E:W, the remaining aqueous fraction was removed by lyophilization. The extraction yield was ~~was~~ calculated as the weight of total material

159 extracted respect to the weight of initial insect sample, and expressed as percentage. The
160 obtained UAE extracts were kept at -20ºC until further use.
161
162 **2.3.2 Pressurized liquid extraction**
163 Extractions were performed using an accelerated solvent extractor (ASE, 350,
164 Dionex Corp, Sunnyvale, CA, USA) equipped with a solvent controlled unit. The
165 amount of 2 g of insect was loaded into the stainless-steel cell with sea sand (thin grain,
166 particle size 250 – 300 μm, Sigma-Aldrich, Madrid, Spain) above and below the sample
167 to avoid any void spaces and 20 mL of solvent were added afterwards. Extractions were
168 performed at 120°C for 15 min and 100 bars, using N_2 as compressor gas. After that,
169 solvent was removed from samples using a rotary evaporator. In the case of samples
170 extracted with E:W, the remaining aqueous fraction was removed by lyophilization. The
171 extraction yield was calculated as the weight of total material extracted respect to the
172 weight of initial insect sample, and expressed as percentage. The obtained PLE extracts
173 were kept at -20ºC until further use.
174
175 **2.4 Fatty acid profile**
176 Analysis of fatty acids (FAs) was performed by GC-FID previous derivatization
177 by transesterification using the method of Miller & Berger (1985). Briefly, 5 mg of
178 sample were saponified with 250 μL of NaOH solution (45 g NaOH in 150 mL of
179 distilled water and 150 mL of methanol) and incubated at 100ºC for 30 min. The sample
180 was then heated in a water bath at 80°C for 20 min with 500 μL of 6 N HCl in methanol
181 to achieve FAs methylation. After cooling, the fatty acid methyl esters (FAMEs) were
182 extracted with a mixture of hexane:anhydrous diethyl ether (1:1, v/v) and centrifuged at
183 14000 rpm for 5 min. Thereafter the lower aqueous phase was discarded, and the

184 remaining upper phase was dried with N_2. The resulted sample was then dissolved in
185 hexane (150 µL) and analyzed by a GC-FID. The equipment was a 7890A System
186 (Agilent Technologies, (Loveland, CO 80537, USA) comprising a split/splitless
187 injector, electronic pressure control G4513A autoinjector and a FID detector. The
188 column employed was an Agilent HP-5MS UI capillary column (30 m × 0.250 mm ×
189 0.25 µm). Helium was used as a carrier gas at a constant flow of 1.8 mL/min. Oven
190 temperature ramp started at 50°C, increased to 210°C at 20°C per min and held for 18
191 min. Then, temperature was increased to 230°C at 20°C per min and held at 230°C for
192 13 min. The injection volume was 1µL in splitless mode.

193 Palmitic, linoleic, oleic, stearic and arachidic acids were identified and quantified
194 with their own calibration curves constructed with commercial standards. Palmitic acid
195 standard was used for the quantification of lauric, myristic, pentadecyl, palmitoleic,
196 margaroleic and margaric acids, and the calibration curve of arachidic acid was used for
197 the quantification of gadoleic and behenic acids. The results were expressed as g of fatty
198 acid/100 g of FAMEs.

199 To know the variability in the fatty acid profile of the extracts versus the initial
200 insect fat, the later was analysed following the same derivatization and analysis
201 procedure. For this purpose, the total lipid fraction of whole insects was extracted
202 following the method of Folch, Lees, & Sloane Stanley (1957). Briefly, 1 g of ground
203 initial insect sample was soaked in 20 mL of chloroform:methanol (2:1, v/v). The
204 mixture was homogenized (Ultra-Turrax IKA T18) for 2 min at 11000 rpm and
205 centrifuged at 3000 rpm for 10 min. The supernatant was collected and mixed with 4
206 mL of water. Then, it was centrifuged at 3000 rpm for 10 min. After that, the upper
207 aqueous phase was removed, and the lower organic phase was dried using a rotary

evaporator. The resulted dried extract was weighted to determine the total fat content, and subsequently, it was submitted to derivatization and chromatographic analysis.

2.5 Evaluation of the nutritional quality of lipids

The nutritional quality of the lipids from both the initial insects and the extracts was assessed by considering the polyunsaturated/saturated fatty acids (PUFAs/SFAs) ratio and two indices related to the risk of coronary disease: atherogenic index (AI) and thrombogenic index (TI) (Ulbricht & Southgate, 1991). The AI indicates the relationship between the sum of the main saturated fatty acids (SFAs) and that of the main classes of unsaturated fatty acids (UFAs), the former being considered proatherogenic, and the latter antiatherogenic (Łuczyńska, Paszczyk, Nowosad, & Łuczyński, 2017). The TI shows the trend to form clots in the blood vessels. This is defined as the relationship between the prothrombogenetic (SFA) and the antithrombogenetic FAs (monounsaturated fatty acids –MUFA–, ω-6 PUFA and ω-3 PUFA) (Łuczyńska et al., 2017; Telahigue, Hajji, Rabeh, Hamed, & Cafsi, 2013; Ulbricht & Southgate, 1991). These indices were calculated based on the profile of the fatty acids of the samples, according to Ulbricht & Southgate (1991):

$$AI = [C12:0 + C14:0 + C16:0] / (\Sigma \text{ MUFA} + \Sigma\ \omega\text{-6 PUFA} + \Sigma\ \omega\text{-3 PUFA})$$

$$TI = [C14:0+ C16:0+ C18:0] / [(0.5 \times \Sigma \text{ MUFA}) + (0.5\times \Sigma\ \omega\text{-6 PUFA}) + (3 \times \Sigma\ \omega\text{-3 PUFA}) + (\omega\text{-3 PUFA}/\ \omega\text{-6 PUFA})]$$

2.6 Cholesterol content

The cholesterol content of both the extracts and the lipid fraction of the initial whole insects was quantified by GC-FID after sample derivation with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA). For this purpose, extracts were dissolved in

BSTFA at a concentration of 20 mg/mL and subsequently, they were incubated at 75°C for 60 min with shaking every 15 minutes. After incubation and cooling, the samples were analysed by the same GC-FID instrument as described in the section 2.4, although the ramp temperature was slightly modified. Oven temperature started at 50°C and was increased at 10°C/min up to 310°C, which was maintained for 25 min. Cholesterol was quantified with a calibration curve of a commercial standard of cholesterol, which was derivatized under the same conditions as the samples.

2.7 Statistical analysis

The statistical analysis was carried out with the general linear model of the SPSS 24.0 software (SPSS Inc., Chicago, IL, USA) through the analysis of variance. Significant differences were considered for values $p \leq 0.05$. The Tukey Post-hoc test was performed to establish significant differences between the mean values.

3. Results and Discussion

3.1 Insect extraction yield

Extraction yields from both insects by either PLE or UAE and using different solvents are shown in Table 1. Regardless of the extraction method and solvent used, *T. molitor* showed higher extraction yield than *A. domesticus* ($p = 0.001$), leading to mean values closer to 30% and 15%, respectively. It is known that some insects have the capacity to accumulate higher quantities of lipids, mainly during the first stages of their development, such as larva or pupa (Paul et al., 2017). This fact could explain the higher yield obtained for *T. molitor*, under the form of larva, compared with *A. domesticus*, under the form of adult. Additionally, it should also be considered that the differences observed in the extraction yield between both insect species might as well

be related to the fact that these insects belong to different biological orders, and their lipid and general composition is different, which might affect the final total amount of extracted material.

Concerning the extraction methods, despite a trend to higher yields was observed by PLE (mean value around 24%) compared to UAE (mean value around 19%), differences were not significant (p = 0.836). Regarding the effect of the extraction solvent, a significant effect of this factor was observed, regardless of the insect and the extraction method (p = 0.040). Thus, in general, the use of E allowed higher extraction yields compared with E:W, showing mean values of around 25% and 18%, respectively. Therefore, comparing all the extracts, the highest extraction yield corresponded to *T. molitor* extracted by PLE with any of the solvents; whereas the lowest extraction yield corresponded to *A. domesticus* extracted by PLE with E:W (Table 1).

3.2 Fatty acid profile of the insect and insect extracts

The fatty acid profiles of both the insects and the PLE or UAE insect extracts, from either *A. domesticus* or *T. molitor* are shown in Table 2. Previously, in case of the insects, their total fat content was estimated. Values were 13.7% for *A. domesticus* and 23.4% for *T. molitor*. These results were similar to those previously described in other studies where the same edible insects were studied, as for example 23.6 to 28% of total fat content for *T. molitor* or 8 to 15% for *A. domesticus* (De Marco et al., 2015; Iaconisi et al., 2017; Paul et al., 2017; Tzompa-Sosa et al., 2014). The different lipid content observed for these insect species was expected, since it has been described that the order Coleoptera, such as *T. molitor*, has generally a higher fat content compared with the order Orthoptera, such as *A. domesticus* (Durst et al., 2010; Kouřimská & Adámková, 2016; Tzompa-Sosa et al., 2014).

When the fatty acid profile of the initial insects was analysed, thirteen fatty acids were identified in different proportions. In general, more than 70% of FAMEs from both insects were unsaturated fatty acids (UFA), such as palmitoleic (C16:1), margaroleic (C17:1), oleic (C18:1), linoleic (C18:2) and gadoleic (C20:1) acids. The remaining content was saturated fatty acids (SFA) including lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0), arachidic (C20:0) and behenic (C22:0) acids. Quantitatively, linoleic was the major fatty acid found in both insects, followed by oleic and palmitic acids.

Concerning specific differences between both insect species, *T. molitor* showed a significantly higher UFA total content compared with *A. domestics* (77.2% and 70.7%, respectively) (Table 2). This difference was mainly due to the oleic acid content, being 34.3% for *T. molitor* and 20.9% for *A. domesticus*. However, it is important to remark that the total PUFAs content was significantly higher in *A. domesticus*, due to the higher content of linoleic acid compared with *T. molitor* (48.0% and 39.9%, respectively). Nevertheless, the total SFA content was also significantly higher in *A. domesticus*, mainly due to the higher palmitic and stearic acid content. Similar fatty acid profiles for both insects were also described in other studies (Paul et al., 2017; Tzompa-Sosa et al., 2014).

Comparing the fatty acid profile between the initial insect and their corresponding extracts, significant changes were observed depending on the extraction conditions for both insects (Table 2). In the case of *A. domesticus*, this difference was only significant when it was subjected to UAE-E:W extraction. Thus, a decrease in the total SFA and MUFA contents and an increase in the total PUFA content was observed in these *A. domesticus* extracts. In fact, the most remarkable enrichment of these extracts was obtained for linoleic acid, changing from 48.0% to 63.7% after the procedure of

309 extraction. On the contrary, these extracts showed decreased levels of palmitic acid and
310 oleic acid compared with the initial fatty acid profile of the insect.

311 In the case of *T. molitor*, there was also a significant increase in the total PUFA
312 content and a decrease in the total SFA and MUFA contents for the UAE-E:W extracts,
313 similar to the *A. domesticus* extracts. Additionally, such PUFA enrichment was mainly
314 due to linoleic acid, being also in agreement with *A. domesticus* extracts.

315 On the contrary, for *T. molitor* and for the rest of extraction conditions (UAE-E,
316 PLE-E:W and PLE-E), a significant decrease of the total PUFA content in the extracts
317 was observed compared with the initial insect, whereas a significant MUFA enrichment
318 of the extracts was detected instead. More specifically, these extracts were enriched in
319 oleic acid. Additionally, these extraction conditions also led to a significant decrease in
320 the SFA content of the extracts, due to palmitic acid decrease.

321 When comparing between extraction methods, that is, PLE or UAE, and without
322 considering the insect or the solvent, no significant differences were observed in the
323 fatty acid profile. On the contrary, when comparing between the extraction solvents, it
324 was observed that the use of E:W resulted in a higher PUFA enrichment and a lower
325 SFA content of the extracts compared with E, but only for the UAE method. This was
326 found for both *A. domesticus* and *T. molitor* extracts. This effect of the solvent might be
327 due to a lower affinity of UFA for medium polarity solvents such as E, in which they
328 are extracted in lower amounts compared with E:W (Dole & Meinertz, 1960). However,
329 no clear effect of the extraction solvent was observed in PLE method.

330 The effect of advanced methods of extraction on the modification of the fatty acid
331 profile of insects has not been previously described, but some similar effects have been
332 described for other matrices. Otero et al. (2018) obtained PLE extracts from algae richer
333 in long chain UFA when they were extracted by ethyl acetate, compared with ethanol or

acetone. Similarly, Castejón & Señoráns (2019) obtained higher omega-3 concentrates from algae thanks to PLE extraction with hexane compared with the traditional method of solid-liquid extraction of lipids. In the present study, it could be concluded that regardless of the insect studied, the UAE-E:W extraction of insects might allow to obtain insect extracts with a relevant enrichment in PUFA, as linoleic acid, compared with the initial insects. On the contrary, only for *T. molitor*, PLE extraction or UAE-E might allow to obtain insect extracts with a relevant enrichment in MUFA, as oleic acid, compared with the initial insect. Therefore, by using these clean and advanced methods of extraction, it might be possible to selectively obtain insect extracts with different fatty acid profiles compared with the initial insects, containing up to more than 80% UFA and less than 20% SFA for both insects.

3.3 Lipid indices of the fatty acid profile of insects and insect extracts

Taking into account the significant modification of the fatty acid profile observed for the extracts from each insect, it was considered of interest to evaluate the magnitude of such modification from the nutritional and health point of view. For this purpose, PUFA/SFA, AI and TI indices were estimated for both the insect extracts and the initial insects. In this respect, it has been suggested that a PUFA/SFA ratio above 1 or 1.5 is linked to a reduction in the risk of cardiovascular disease (Berasategi et al., 2011; Czernichow, Thomas, & Bruckert, 2010), and the lower the AI and TI indexes, the lower the risk of the development of cardiovascular disorders (Attia, Al-Harthi, Korish, & Shiboob, 2015).

As shown in Figure 1, the initial insects from *A. domesticus* and *T. molitor* showed a PUFA/SFA ratio above 1.5 for both species. Additionally, the IA and IT values of both insects were considered low, although those from *T. molitor* were the

lowest. Therefore, the fatty acid profile of *T. molitor* might be considered healthier than that of *A. domesticus*, mainly due to a lower level of total SFA and higher level of total MUFA (Table 2).

Concerning the insect extracts, it is important to remark that despite the significant modification of the fatty acid profile previously described for most insect extracts (Table 2), most of them showed the same lipid indices than those of their corresponding initial insect (Figure 1). This would suggest that the general nutritional and health properties of their fatty acids might be the same. However, the values of the lipid indices for the UAE-E:W extracts from both species were significantly different compared with their corresponding initial insects. As shown in Figure 1, under UAE-E:W extraction, the PUFA/SFA ratio increased closer to 3 for both insect extracts. This was mainly due to the previously described enrichment in linoleic acid obtained under these conditions of extraction for both species of insect extracts.

In order to evaluate the magnitude of the PUFA/SFA values of the UAE-E:W extracts, these values were compared to those from usual dietary oils and fats. To do that, the lipid indices of either animal fats, vegetable oils or fish oils were estimated from their corresponding fatty acid profile reported in the scientific literature (Gunstone, Harwood, & Dijkstra, 2007; O'Brien, 2009). As shown in Figure 2.a, the PUFA/SFA value of both initial insects and their UAE-E:W extracts showed PUFA/SFA indices in between fish oils and high PUFA vegetable oils, but the index of the insect extracts tended to be closer to vegetable oils than fish oils when compared with the index of the initial insects.

Concerning the AI and TI indices, these values significantly decreased for the UAE-E:W extracts compared with their initial insects (Figure 1). These values were also compared to those estimated from the fatty acid profile of dietary oils and fats

(Gunstone et al., 2007; O'Brien, 2009). As shown in Figure 2.b, in the case of the lipid indices from both the initial insects, it was found that the values were very similar to those of other animal fats, namely poultry fats, such as chicken fat or goose fat. On the contrary, in the case of both UAE-E:W extracts, the indices distanced from those of animal fats and tended to be in between those of poultry fats and vegetable oils, especially in the case of *T. molitor* extracts.

Therefore, it was concluded that the extraction of insects matrices by UAE-E:W might allow to produce insect extracts with different and improved fatty acid profile from the health point of view compared to the initial insect fat.

3.4. Cholesterol content of the insects and insect extracts

As for most animals, it has been described that insects contain relevant levels of cholesterol within their tissues (Ramos-Bueno et al., 2016; Rumpold & Schlüter, 2013; Tzompa-Sosa et al., 2014). Therefore, due to the important implications of cholesterol in cardiovascular diseases, and taking into account that the extraction procedures might lead to modifications in the general lipid profile, the evaluation of the cholesterol content of both insect species and their corresponding extracts was considered of interest.

As shown in Figure 3, the initial insects showed a cholesterol content closer to 0.1 g/100 g of dry insect, but *T. molitor* showed a trend to higher cholesterol levels than *A. domesticus*. These values were similar to those described for these insects by Ramos-Bueno et al. (2016).

When the insect matrices were submitted to extraction, a general cholesterol enrichment was observed for all the extracts. However, this enrichment was significantly higher for the *A. domesticus* extracts than for *T. molitor* extracts, regardless

409 of the method and solvent of extraction (p = 0.028) (mean values 0.39 ± 0.09 g/100 g
410 and 0.30 ± 0.03 g/100g, respectively). The explanation of this result is complex,
411 considering the great initial differences between *A. domesticus* and *T. molitor*
412 concerning aspects such as their different development state (larval vs adult), the initial
413 fat content, or the cuticular surface. However, the obtained result would suggest that the
414 cholesterol-enrichment of insect extracts would be specie-dependent.

415 Concerning the method of extraction, despite PLE extracts seemed to reach a
416 higher concentration of cholesterol compared with UAE extracts (mean values 0.37 ±
417 0.06 g/100 g and 0.33 ± 0.09 g/100g, respectively), a lack of a significant effect of the
418 method of extraction on cholesterol levels was found (p = 0.366). Attending the
419 extraction solvent, E extracts significantly favoured the concentration of cholesterol
420 within the extracts, regardless of the insect and the method of extraction (p = 0.018)
421 (mean values 0.39 ± 0.08 g/100 g and 0.30 ± 0.05 g/100g, for E extracts and E:W
422 extracts, respectively). This effect might be expected since the lower polarity of the E
423 solvent might favour the extraction of cholesterol, compared with the higher polarity of
424 the E:W solvent mixute. Therefore, when all the extracts were compared, the lowest
425 cholesterol concentration was observed for UEA-E:W extracts from both insect species
426 (0.27 g/100 g) (Figure 3). In this respect, it is important to remark that these last
427 conditions of extraction were also those that led to the most interesting fatty acid profile
428 from the health point of view (Figure 1). Therefore, it might be concluded that any
429 extraction procedure of insects might lead to extracts with higher cholesterol content
430 than the original matrices, although the UAE-E:W conditions are preferred due to the
431 lowest cholesterol concentration and the best fatty acid profile of the extracts, regardless
432 of the insect.

Finally, it is important to remark that despite the observed effect of cholesterol concentration of the extracts, the total expected intake of cholesterol through the extracts might be low. This is because, as most of natural extracts, their intended uses might be as nutraceuticals or minor ingredients to be included within food matrices, so the expected amount of extracts to be ingested as part of a ration, and hence cholesterol intake per ration, would be low.

4. Conclusions

Extracts from the edible insects *T. molitor* or *A. domesticus* can be obtained by clean and advanced methods of extractions, such as PLE or UAE, being the extraction yield mainly conditioned by the own species nature and the solvent of extraction.

Linoleic acid is the major fatty acid found in both insect extracts, followed by oleic and palmitic acids, but, in general, the fat of *T. molitor* is more unsaturated compared with *A. domestics*. Regardless of the species, this study shows that insect extracts with a modified fatty acid profile compared with the initial insects can be selectively obtained by advanced methods of extraction. Especially, UAE-E:W conditions are preferred if a PUFA enrichment or SFA decrease is sought. In fact, these conditions of extraction lead to insect extracts with healthier lipid indices compared with the initial insects. Additionally, despite any procedure of extraction leads to a cholesterol enrichment of the extracts, the UAE-E:W condition causes the lowest cholesterol concentration.

Therefore, this study shows that insect extracts might be an additional way to impulse other alternative presentations of insect-based foods for human consumption, with the additional advantage of obtaining improved insect ingredients from a lipid profile point of view when compared to the raw insects. Additional studies are currently

458 being carried out in order to characterize the extracts for other minor compounds, as
459 well as potential bioactive effects of interest.
460
461 **Abbreviations used**

E	Ethanol
E:W	Ethanol:Water
FAME	Fatty Acid Methyl Esters
MUFA	Monounsaturated Fatty Acids
PLE	Pressurized liquid extraction
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
UAE	Ultrasound-assisted extraction
UFA	Unsaturared Fatty Acids

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468
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470 Declaration of interests: none.
471
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575

Table 1. Extraction yield of insects (g extract/100 g dry insect)[a]

	PLE		UAE	
	E	E:W	E	E:W
Acheta domesticus	24.85 ± 1.53 [b]	5.02 ± 2.36 [d]	15.48 ± 0.16 [c]	15.05 ± 0.01 [c]
Tenebrio molitor	32.37 ± 1.15 [a]	33.87 ± 2.97 [a]	28.85 ± 1.45 [ab]	17.14 ± 0.19 [c]

[a] Results are shown as mean ± standard deviation (n = 2). Different letters among all extracts mean significant differences ($p \leq 0.05$)

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577

578

Table 2. Fatty acid profile (g/100 g FAMES) of the insect and insect extracts

	Acheta domesticus					*Tenebrio molitor*				
		PLE		UAE			PLE		UAE	
FAME	Insect	E:W	E	E:W	E	Insect	E:W	E	E:W	E
12:0	0.07 ± 0.00	0.10 ± 0.00 [a]	0.10 ± 0.02 [a]	0.02 ± 0.02*[b]	0.09 ± 0.03[a]	0.19 ± 0.03[†]	0.25 ± 0.05 [ab]	0.32 ± 0.06* [a]	0.15 ± 0.06 [b]	0.21 ± 0.03 [ab]
14:0	1.28 ± 0.03	0.50 ± 0.04*	0.52 ± 0.02*	0.88 ± 0.28	0.38 ± 0.02*	4.05 ± 0.04[†]	2.90 ± 0.11* [b]	2.73 ± 0.18* [b]	4.86 ± 0.24* [a]	2.57 ± 0.11* [b]
15:0	0.17 ± 0.02	0.06 ± 0.01* [b]	0.14 ± 0.01 [a]	0.06 ± 0.01* [b]	0.16 ± 0.02 [a]	0.12 ± 0.04[†]	0.16 ± 0.03	0.30 ± 0.15	0.15 ± 0.02	0.17 ± 0.04
16:0	22.84 ± 0.27	22.41 ± 0.40 [ab]	22.99 ± 0.88 [a]	17.20 ± 2.94* [b]	23.44 ± 0.24 [a]	16.66 ± 0.09[†]	14.46 ± 0.98 [a]	15.71 ± 0.26 [a]	10.96 ± 1.54* [b]	15.29 ± 1.61 [a]
16:1	1.69 ± 0.07	1.28 ± 0.05 [ab]	1.34 ± 0.02 [ab]	1.65 ± 0.33 [a]	0.92 ± 0.06* [b]	3.01 ± 0.11[†]	2.11 ± 0.17* [b]	2.16 ± 0.11* [b]	2.83 ± 0.03 [a]	1.86 ± 0.10* [b]
17:0	0.13 ± 0.02	0.12 ± 0.01 [a]	0.13 ± 0.03 [a]	0.04 ± 0.01* [b]	0.12 ± 0.01 [a]	0.11 ± 0.06	0.12 ± 0.02	0.14 ± 0.07	0.06 ± 0.00	0.10 ± 0.01
17:1	0.05 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.17 ± 0.01[†]	0.18 ± 0.03	0.09 ± 0.02	0.13 ± 0.04	0.11 ± 0.02
18:0	4.65 ± 0.12	4.83 ± 0.01 [b]	6.47 ± 0.06* [a]	2.31 ± 0.70* [c]	6.13 ± 0.34* [ab]	1.57 ± 0.04[†]	1.59 ± 0.09	2.03 ± 0.28	1.18 ± 0.56	2.04 ± 0.19
18:1	20.91 ± 0.24	20.69 ± 0.25 [b]	22.23 ± 0.09* [a]	14.03 ± 0.63* [c]	22.39 ± 0.12* [a]	34.26 ± 1.26[†]	40.24 ± 0.81* [a]	39.80 ± 1.21* [a]	28.08 ± 1.34* [b]	42.01 ± 0.52* [a]
18:2	48.00 ± 0.39	49.74 ± 0.74 [b]	45.71 ± 0.90 [b]	63.65 ± 3.31* [a]	45.96 ± 0.39 [b]	39.85 ± 1.23[†]	37.85 ± 1.56 [b]	36.58 ± 1.35* [b]	51.51 ± 0.71* [a]	35.56 ± 0.86* [b]
20:0	0.15 ± 0.02	0.18 ± 0.00 [b]	0.29 ± 0.01* [a]	0.11 ± 0.04 [b]	0.29 ± 0.03* [a]	0.05 ± 0.01[†]	0.08 ± 0.01	0.11 ± 0.02*	0.08 ± 0.01	0.10 ± 0.02*
20:1	0.04 ± 0.01	0.05 ± 0.00 [b]	0.09 ± 0.02* [a]	N.D.* [c]	0.07 ± 0.01* [a]	0.04 ± 0.01	0.07 ± 0.01 [a]	0.08 ± 0.02 [a]	N.D.* [b]	0.07 ± 0.02 [a]
22:0	0.03 ± 0.00	N.D.*	N.D.*	N.D.*	N.D.*	0.01 ± 0.00[†]	N.D.*	N.D.*	N.D.*	N.D.*
ΣSFA	29.33 ± 0.22	28.18 ± 0.43 [a]	30.62 ± 0.84 [a]	20.61 ± 2.78* [b]	30.62 ± 0.23 [a]	22.75 ± 0.10[†]	19.56 ± 0.88* [ab]	21.33 ± 0.62 [a]	17.44 ± 0.69* [b]	20.46 ± 1.31* [a]
ΣMUFA	22.68 ± 0.18	22.08 ± 0.31 [b]	23.74 ± 0.07 [a]	15.74 ± 0.64* [c]	23.42 ± 0.16 [a]	37.39 ± 1.27[†]	42.59 ± 0.70* [a]	42.09 ± 1.14* [a]	31.04 ± 1.36* [b]	44.01 ± 0.48* [a]
ΣPUFA	48.00 ± 0.39	49.74 ± 0.74 [b]	45.71 ± 0.90 [b]	63.65 ± 3.31* [a]	45.96 ± 0.39 [b]	39.85 ± 1.23[†]	37.85 ± 1.56 [b]	36.58 ± 1.35* [b]	51.51 ± 0.71* [a]	35.56 ± 0.86* [b]

The results are expressed as mean ± standard deviation (n = 3). N.D.: not detected.

[†]: Significant differences between the two insects ($p \leq 0,05$).

[*]: Significant differences between each insect and its corresponding extracts ($p \leq 0,05$).

Mean values with different letters within each row of insect extracts within the same insect are significantly different ($p \leq 0,05$).

579

580

581 **Figure Captions**

582

583 **Figure 1.** Lipid indices of *A. domesticus* (A) and *T. molitor* (B) insects and their
584 extracts obtained by the four different extraction conditions. Values with different
585 letters within the same index are significantly different ($p \leq 0.05$) ($n = 3$).

586

587 **Figure 2.** Comparative fatty acid indices (a. PUFA/SFA, b. TI and AI) of insects, UAE-
588 E:W insect extracts and typical dietary fats.

589

590 **Figure 3**. Cholesterol content (g/100 g) of insects and insect extracts.

Figure 1.

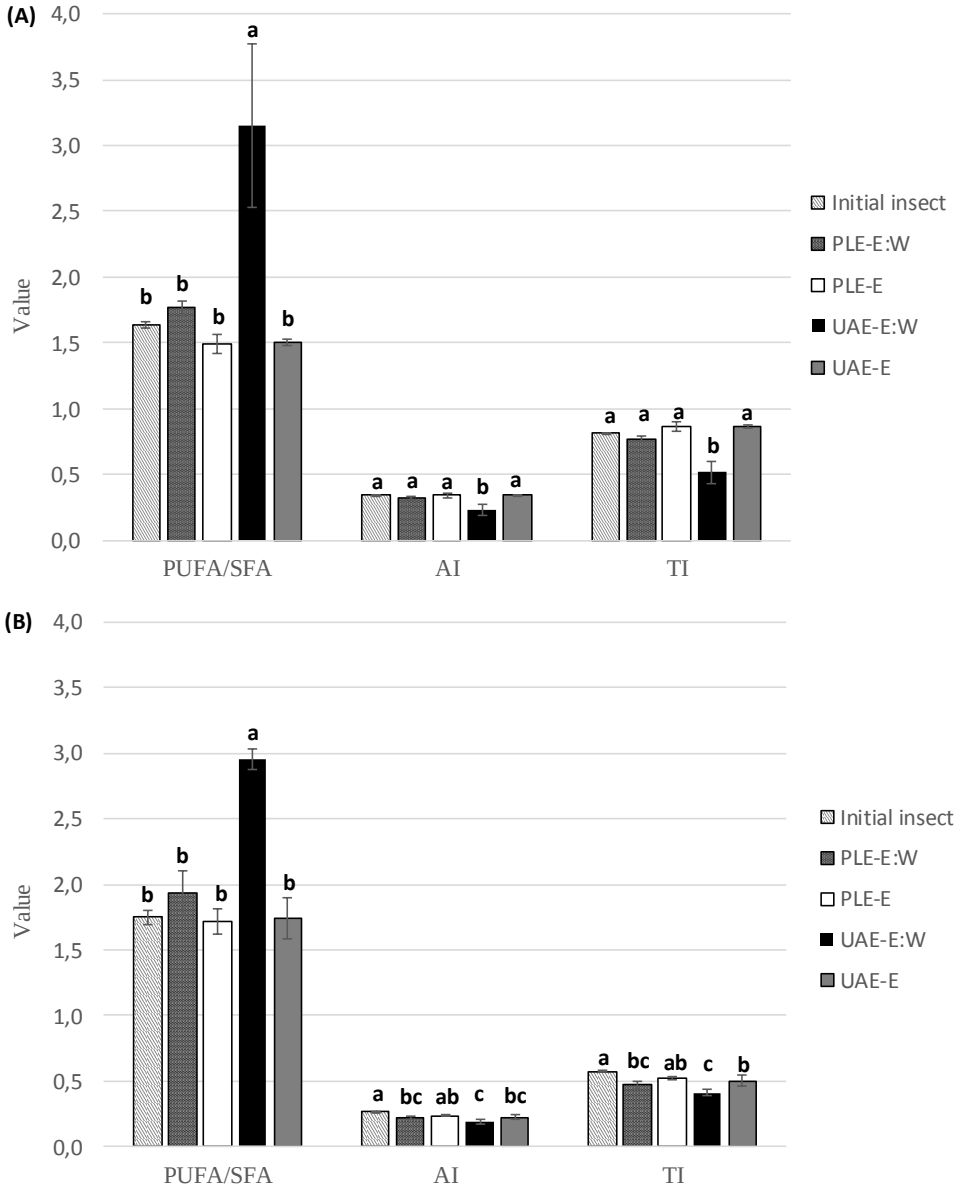
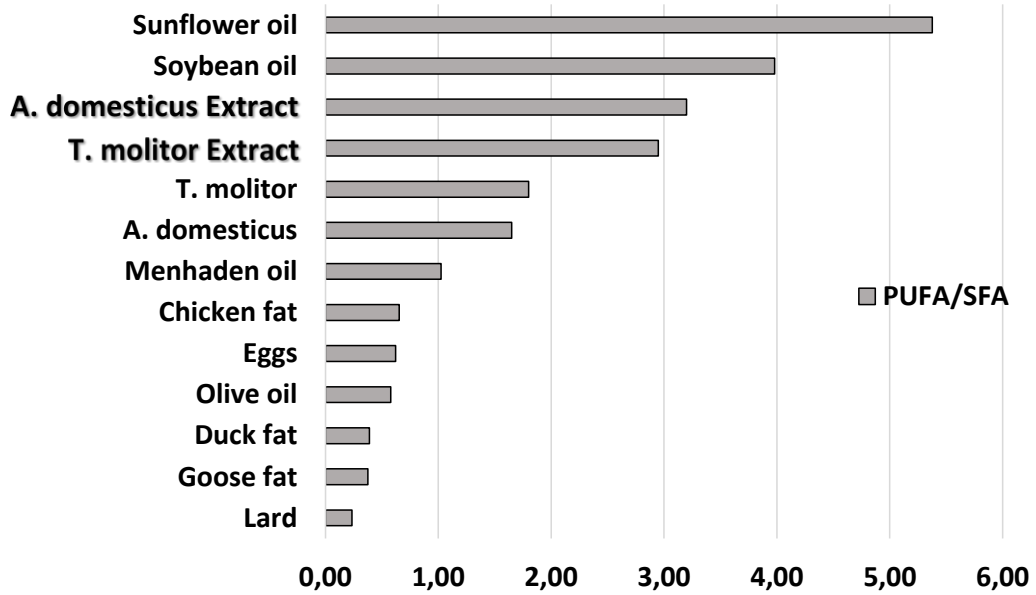
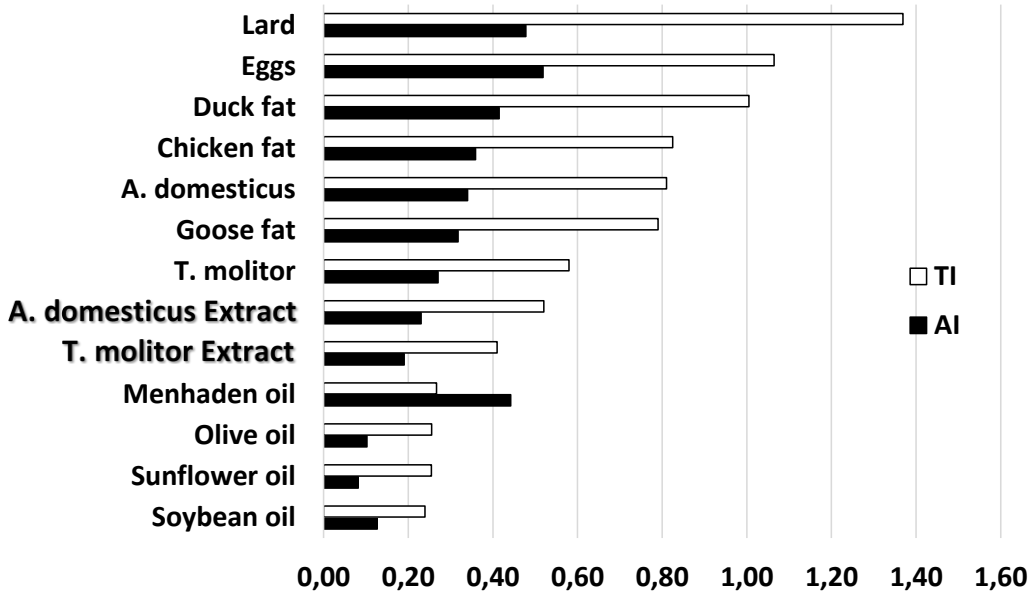

(A)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Value
a
b b b b
a a a b a
a a a b a
PUFA/SFA
AI
TI
Initial insect
PLE-E:W
PLE-E
UAE-E:W
UAE-E
(B)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Value
a
b b b b
a bc ab c bc
a bc ab c b
PUFA/SFA
AI
TI
Initial insect
PLE-E:W
PLE-E
UAE-E:W
UAE-E

Figure 2.

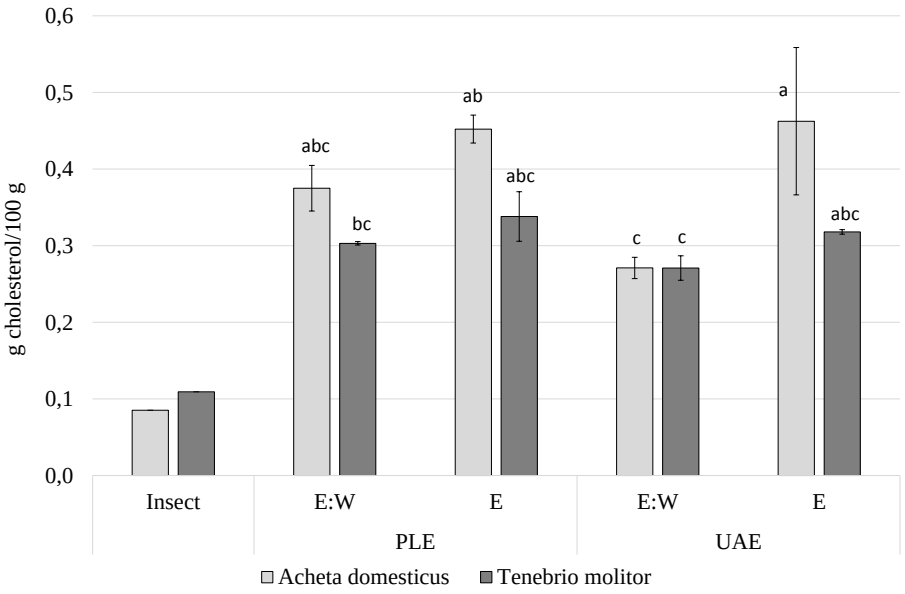
a)


Sunflower oil
Soybean oil
A. domesticus Extract
T. molitor Extract
T. molitor
A. domesticus
Menhaden oil
Chicken fat
Eggs
Olive oil
Duck fat
Goose fat
Lard
PUFA/SFA
0,00
1,00
2,00
3,00
4,00
5,00
6,00

b)


Lard
Eggs
Duck fat
Chicken fat
A. domesticus
Goose fat
T. molitor
A. domesticus Extract
T. molitor Extract
Menhaden oil
Olive oil
Sunflower oil
Soybean oil
TI
AI
0,00
0,20
0,40
0,60
0,80
1,00
1,20
1,40
1,60

598 **Figure 3.**



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